

METHOD AND APPARATUS FOR DETECTING ENZYMATIC ACTIVITY USING MOLECULES THAT CHANGE ELECTROPHORETIC MOBILITY

Abstract of the Disclosure

5 The activity of intracellular chemical reactions of molecules is measured by the use of fluorescently labeled substrate molecules that undergo a change in electrophoretic mobility upon chemical reaction such as that catalyzed by an enzyme. Specificity is achieved by using labeled substrate molecules that can be acted upon only by specific enzymes. Thus the activity of a specific enzyme or class of enzymes can be determined. Measurements are made with the intracellular presence of such substrate molecules, at some time of interest, typically after exposure of the cell to a stimulus that activates a particular enzymatic pathway. To ensure accuracy, measurements must be made in a timely manner so as to minimize chemical reactions occurring subsequent to the time of interest. Fast controllable laser lysis is used to obtain the contents of a single cell into which reporter substrate molecules have been introduced. The cell contents are then subjected to capillary electrophoresis and enzymatic activity is determined by comparing amounts of substrate molecules to amounts of enzymatically altered substrate molecules which are separated by the electrophoresis and identified by the presence of a fluorescent label.

15